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SOME NEW EVIDENCES FOR THE INDIVIDUALITY OF THE CHROMOSOMES.

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INTRODUCTION.

Owing to the renewed interest in hybridization aroused by the rediscovery of Mendel's laws, any facts that throw light on any of the theories used to explain the complex phenomena of the inheritance of specific characters in crosses must be welcome to biologists. In his résumé of the observations on hybrids and germ-cell generation Haecker (13) lays great stress on the theory of the individuality of the chromosomes. The same has been done by Sutton (31), Cannon (7), (8) and others. In this paper I propose to publish some observations made on the germ cells of crickets which furnish two lines of evidence establishing still better the individuality of the chromosome.

The first of these concerns the accessory chromosome in whose behavior I have found additional proof of its distinctness from the other chromosomes.

The second line of evidence concerns the ordinary chromosomes. Boveri (5) has recently found a difference *in function* in the chromosomes; Sutton (30) has found a difference *in size;* and I have been fortunate enough to find a difference *in form*, a characteristic shape assumed by the chromosomes in the prophase and metaphase of the first spermatocyte division.

METHODS AND MATERIAL.

As indicated in my former paper (3), Flemming's strong chromo-aceto-osmic fixative is best for the study of cricket germ-cells. Thirty-five per cent. alcohol saturated with corrosive sub-

limate plus ten per cent. acetic acid proved fairly good but material thus fixed shrank too much during imbedding.

The best stains were Heidenhain's iron-hæmatoxylin and Flemming's triple stain.

Besides the material used before, I have collected the common black field cricket about Chicago, and Woods Holl, Mass. The specimens from Lawrence, Kans., were called *Gryllus assimilis* after comparing them with specimens in the University collection there. Mr. F. E. Lutz called the same specimens *Gryllus luctuosus* after comparing them with forms about Chicago. I shall not enter into a discussion as to what are good species among *Gryllus* or what species are found in the different localities, but shall continue to use the name *Gryllus assimilis* for the species of the common black field cricket until the species are better differentiated.

The testes of the field cricket were described in the former paper and that of *Gryllus domesticus* corresponds very closely with it as to shape, size, location and arrangement of follicles and cysts.

The number of chromosomes in the field cricket is twenty-nine in the spermatogonia and not twenty-three as suggested in the first paper. Then I determined the number by counting the chromosomes in the equatorial plate of the first spermatocyte division and there the counting is difficult for the reason that the chromosomes usually do not enter the equatorial plate at the same time. I have made a great many counts since both in spermatogonia and spermatocytes and feel confident that the numbers are twenty-nine, and fourteen or fifteen respectively. In *G. domesticus* there are but twenty-one in the spermatogonia and ten or eleven in the spermatocytes. Because of this smaller number *G. domesticus* is more favorable for making drawings.

The stages with their relation and limits and the use of terms is indicated in the former paper. One point stated there I wish to emphasize as it is even more marked in *G. domesticus* than in *G. assimilis*, namely the fact that the cells in one cyst are not all in *exactly* the same stage of development. Some of the cells, usually on one side of the cyst, are a little in advance and others lag a little. This makes it possible to see the exact sequence of stages by comparing the cells of the cysts in different follicles.

Thus one cyst will have most cells in the metaphase but a few will be in early anaphase, while the neighboring cyst in the next follicle may have most of the cells in late anaphase but a few will be in early anaphase. The precocious cells in one cyst connect directly with the laggards of another. This is a great advantage for correct interpretation of the appearances.

Observations.

The accessory chromosome appears in the earliest secondary spermatogonial divisions. During mitosis it takes its place in the periphery of the equatorial plate (Figs. 1 and 2). It always has a bend at the center, and the whole is in the shape of an irregular horseshoe (Fig. 1) or its two ends are spread out almost straight so that it is a straight rod with a short semicircle-like bend at the center (Fig. 2). As the other chromosomes split and pass to the poles the accessory also splits longitudinally (Fig. 3). The curves of the horseshoe-like rods separate first and the ends of the two daughter chromosomes are the last part to be separated, as indicated by McClung (16), in *Xiphidium*. All through the metaphase and anaphase the accessory is quite a little behind the others. Its ends are sometimes scarcely separated when the other chromosomes have reached the poles. During the rest stage it does not become granular and disappear like the others but remains a darkly staining mass.

In the early growth period while the other chromosomes become diffuse, the accessory takes its position against the wall of the nucleus. Here it lies a strongly staining mass adherent to the one side. Through all the long growth period the accessory can be distinguished from the nucleolus by its stain for a part of the time (Fig. 10), but it can be recognized *all the time* by its position as it lies flattened against the outer wall. The nucleolus, which for a part of the growth period takes the same stains as does the accessory and just as readily, can usually be distinguished by its rounded or oval shape. Its position in the nucleus varies with almost every cell. It may be in the center or near the periphery, opposite the accessory (Figs. 4, 5 and 8) or near to it (Figs. 6 and 7). It often lies against the accessory or even partly over it (Figs. 6 and 10). This fact led

Voinov (32) to describe the two closely approximated bodies as a double nucleinic body — "corps nucleinien double." (For a criticism see in "Discussion" below.) I could note no difference in the size of the nucleolus that was constant. In the prophase of the first spermatocyte division the nucleolus gradually loses its ability to stain and disappears completely while the chromosomes are forming.

The accessory chromosome can be distinguished from the ordinary chromosomes in late prophase by its denser stain and smooth outline. But as the chromatin concentrates more and more in the latter we must find other criteria. The position no longer answers, as many of the ordinary chromosomes now come to lie in or near the periphery of the nucleus, and in a few cases it becomes something of a puzzle to know which is the accessory. But about this time the shape assumed by the latter becomes a mark of distinction from the others. As shown in Figs. 12, 13, 15, 17, and 20 it assumes a sausage shape and keeps it all through the first spermatocyte division. As the spindle is formed and the other chromosomes are drawn toward the equatorial plate, the accessory may be in any position whatever within the nucleus. McClung (18) says it appears nearer one pole. It does very frequently. It may be within the spindle or out in the cytoplasm as Sinéty (28) thought he found it in other Orthoptera. But it may lie even in the equatorial plate where I have seen it in several cases. Either the open or the curved side of "the sausage" may point toward its nearer pole. In metaphase and anaphase it is usually the curved side.

As the other chromosomes move to the poles the accessory also seems to be drawn towards its nearer pole but it is again a laggard (Fig. 23). It does not divide but passes entire to one of the poles as Sinéty has already indicated for *Gryllus campestris* and he and McClung have described it in Locustids and Phasmids. I found a few cases where the accessory was left in the plane of cleavage between the two daughter cells. Here it was divided into two equal parts as seen in several cases (Fig. 22), and unequal in one case. In one case the accessory was just in the center between two poles and showed constrictions at its center (Fig. 21). In these cases I believe the accessory happened

to lie in the region of the equatorial plate and neither pole was able to attract it, and so being left in the region of the new cell wall was constricted mechanically. I do not think that this division is the same as that of normal cleavage of the accessory as it occurs in the second spermatocyte division.

The following fact in the behavior of the accessory has not been recorded heretofore by any investigator as far as I know. It throws some additional light on its own independence and hence on the individuality of the chromosomes.

As recorded by most writers on insect spermatogenesis there is no resting stage or no *true* resting stage between the first and the second spermatocyte divisions. But I have found in *Gryllus* what I shall call a *semiresting stage* following Katharine Foot's (11) terminology. She has described a stage in the *Allolobophora* egg to which my own findings correspond in many particulars. At the close of the anaphase of the first division the chromosomes are crowded around the pole. The centrosome divides about the time or a little before the chromosomes reach the pole. The two centrosomes begin to move apart with the radiating fibers of the asters around them individually and the spindle fibers connecting them (Figs. 23 and 24). As the spindle is elongating the chromosomes become somewhat vesicular (Figs. 25 and 26) and have a nuclear wall formed around them, entirely around or only part way around (Figs. 26 and 27). The diffusion of the chromatin and the formation of the nuclear wall seems to go farther in some cells than in others; Fig. 27 shows as much diffusion as any observed. The whole semiresting stage must be very brief for a single cyst may show cells as far apart in development as shown in Figs. 24 and 30. As the chromosomes enter the equatorial plate of the second division they are usually so crowded that it is impossible to count them or distinguish the accessory from the others.

After this brief description of the semiresting stage let us return to the accessory chromosome.

In the semiresting stage the accessory does not enter into the nucleus but forms its own wall around itself. It becomes vesicular, the chromatin becoming granular and showing vacuoles (Figs. 24–27). Its position with relation to the nucleus varies.

Very frequently it lies just outside the nucleus so that there seems to be but one dividing membrane between the two (Fig. 25). It may be entirely away from the nucleus showing the two walls very distinctly (Fig. 26). In one case I found the spindle between the accessory vesicle and the nucleus (Fig. 24). In other cases the accessory lies entirely in or partly in the other nucleus (Fig. 27); but in every case *it has its own vesicular wall around itself, and does not form part of the nucleus.* Of course, the accessory is found in only one half the cells.

When the second spermatocyte spindle is formed the accessory enters the equatorial plate and can not always be distinguished because of the crowding of the chromosomes spoken of above (Figs. 28 and 29). In a few cases I could distinguish the individual ones and count them (Fig. 31). Here the accessory is marked by its size and characteristic shape. But as soon as all the chromosomes divide and move apart in anaphase, they can be counted in almost every cell. The accessory again lags behind but usually not so much as in the earlier stages. Figs. 32 and 33 give side views and Figs. 34 and 35 almost polar views of the anaphase stage; Figs. 32 and 34 show the accessory and Figs. 33 and 35 lack it. Figs. 34 and 35 are very interesting because they were found side by side in a cyst and indicate the absence of the accessory in one half and its presence in the other half of the cells. The accessory in the telophases and in the spermatids has been described in my former paper to which readers are referred for its farther history.

The Ordinary Chromosomes.

While the above description of the accessory applies equally well to either species of *Gryllus*, what follows applies only to *G. domesticus*. *G. assimilas* shows some differences of size and shape in the ordinary[1] chromosomes, but it is not at all marked.

[1] At first I was inclined to adopt Montgomery's (26) terms homochromosome and heterochromosome to distinguish between the ordinary chromosomes and the accessory. But upon reflection it seemed to me it would lead to confusion with the use of the words heterotypic and homotypic. If the terms were adopted then we should have both homochromosomes and heterochromosomes in the homotypic division as well as in the heterotypic. This mixing of terms seems undesirable.

There is here one frequently showing an L shape somewhat as Sinéty (28) has described in a Phasmid, but this is not the accessory joined to an ordinary chromosome as Sinéty interpreted his figure.

In *G. domesticus* in the spermatogonia there are twenty ordinary chromosomes as shown in Figs. 1 and 2. These show differences of size and also in amount of curving. The short ones are straight or almost so, and the long ones are curved, some more, some less. The amount of curving does not depend on the length only as can be seen by comparing chromosome pairs 5 and 6 in Fig. 2. The chromosomes can be arranged into a graded series of pairs following Sutton (30) and Montgomery (25) but the differences between certain pairs is often very slight so that one could often as well make groups of three's or four's. In Fig. 2 I have made an attempt to bring together the probable pairs, but in many cases the arrangement is very unsatisfactory. Of course, looking at the chromosomes themselves with adjusting focus relations are more apparent than can be indicated by a camera drawing. In pairs one and two of Fig. 2 the two chromosomes on the right side of the respective pairs may belong together to form one pair, and the two on the left the other. Grouping them as suggested would bring together chromosomes that do not differ more than do some of the chromosomes of the pairs as they now stand, *e. g.*, pair 3.

After the spermatogonial divisions are completed the cells enter the growth period. The chromosomes seem at first to break up and the chromatin apparently becomes diffuse, yet it appears partly in threads as shown in Fig. 4. I have looked very long and carefully for a massing of the chromatin thread into one part of the nucleus hoping thus to find what Montgomery (22) had described as the "synapsis stage" I was unable to find anything that corresponded with his description or drawings, although he named *Gryllus* as one of the forms in which he saw the synapsis stage. But upon studying the ovocytes during the growth period I found many cells showing the conditions indicated in Fig. 36. This drawing is made from an ovary taken from *Scapteriscus didactylus*, a Porto Rican mole cricket, kindly sent me by O. W. Barrett, of the agricultural experiment station. Both

species of *Gryllus* show the same conditions. Many of the other cells show the loops more crowded, especially the younger (smaller) ovocytes while the older (larger) ones show them looser.

With the idea in mind that the chromatin thread is in loops I succeeded in finding Fig. 5 in the early spermatocytes. This figure shows the loops as much massed leaving as much of the nucleus clear as any case I have observed. In most cells the loops fill the nucleus completely ; besides the large majority of the cells are so placed in the field that they show nothing of the looping of the thread. This I consider the characteristic of the "synapsis stage" in the testis of *Gryllus* and not the massing to one part of the nucleus as Montgomery did in his work on *Pentatoma*.

This stage appears quite early in the growth period and lasts a comparatively long time. All the cysts from which Figs. 5, 6, 7 and 8 are drawn show some cells where the loops of the chromosomes are quite evident, but most cells show little or nothing of such loops owing to the plane of cutting, as indicated in the figures.

After this stage the chromatin becomes a little more diffuse again before forming the definite chromosomes of the first spermatocyte division (Fig. 10). We shall now pass to the metaphase where the chromosomes are completely formed and lie in the equatorial plate region and return to the late prophases subsequently.

A glance at Fig. 18 will give the reader an idea of the various forms of chromosomes seen in a cyst full of cells in this stage. Drawings of all the forms that could be found showed the possibility of classifying the shapes into rings, crosses and rods. The rod may be straight, or bent so as to form a bracket, parenthesis or an *S*. The crosses may have the ends bent so as form an *f* or an ϵ where one arm is very short or absent. All these shapes can be seen in Figs. 13–19. It was observed that many cells showed two rings (Figs. 13 and 18, *a*) and one or two crosses and several rods some of which were straight. If there were many straight rods fewer other shapes appeared. Some showed no rings others no crosses. Naturally the question arose : is there any constancy in the number of chromosomes in a cell that assume a certain shape?

To test this I sketched twenty-one groups of chromosomes that showed a variety of shapes and classified them into rings, crosses, rods, brackets, parenthesis, *f*'s, *S*'s and ϵ's. I found that but two groups showed no rings and eleven groups showed two rings, but in no case were there more than two. Five cases showed no crosses and nine cases showed two.

It is evident that any one of the shapes represented may be turned in such a way in the cell that it would appear as a straight rod. This might account for the non-appearance of the rings, or any of the other forms in my sketches. I then tried the hypothesis that there were *two rings* in each cell and tested it as follows:

To find out what per cent. of rings should appear I proceeded thus. The ring is formed of a cylindrical rod. Measuring a number of rings with the eye-piece micronometer I found that the opening was about one third of the diameter. I then moulded a ring of modeling clay making the rim of a rod whose diameter was equal to that of the central opening. By turning this doughnut-like ring on a sheet of paper it was found that the opening was not visible for about 67° of the semicircle. Sixty-seven and one half degrees is three-eighths of a semicircle, hence three rings out of every eight should not show any opening. But as the ring becomes wide enough to be distinguished as a ring before the opening can be seen, it seemed possible to estimate the percentage of rings that should be recognizable. By using the above ring of clay and testing several of the co-workers in the laboratory as to the angle at which they could be reasonably sure that it was a ring and not a rod they were seeing it was found that on an average the ring could not be recognized for fourty-five degrees of the semicircle. Fourty-five degrees is one-fourth of one hundred and eighty degrees, or one ring in four should not be recognizable.

Of the twenty-one sketched groups ten showed all the ordinary chromosomes. If there are two rings in each cell, according to the hypothesis there should be 20 present, of which 15 should be recognizable. My table shows 13.

I then sketched 25 cells, all showing the full number of chromosomes, 10. These should have 50 rings, three-fourths of which, or 37½, should appear. After completing my table I

counted the number of rings and found 35 with six marked doubtful. If we count one half of these we get 38, or just the required number. This does not prove that there are always two chromosomes that assume the ring shape, but it makes it probable.

A few facts can be added from the prophase stages that increase the probability that these shapes are characteristic of the individual chromosomes. Fig. 11 is a careful drawing made of a cell in a late prophase. The nuclear wall is still intact. The accessory is densely stained and smooth in contour. The ordinary chromosomes are not yet compact or smooth in outline, but they show many of the shapes appearing later. Counting the chromosomes marked *6*, which was drawn from the next section and carefully identified as belonging to this cell, there are the two rings, two crosses and several curved and bent rods. Fig. 8 shows a well-formed ring in an earlier stage. The formation of the tetrads has not been studied in minute detail, since this has been done by McClung in the Acrididæ (17) and Locustidæ (18), more favorable material for this part of the problem. Fig. 9 shows the chromatin rod with the longitudinal split indicated.

Fig. 12 gives a polar view. The nuclear wall has just broken down and the chromosomes have not been drawn into the equatorial plate. Here both ring chromosomes are visible which is unusual for a polar view. Some of the other shapes can be seen. I think that the chromosomes are not yet drawn by the fibers and hence show these shapes from this view. Figs. 13, 14 and 16 and *a*, *b*, *c* in 18 show the diversity of forms that can be made out in a single nucleus. The varied shapes can best be seen in a nucleus just after the nuclear wall has broken down and the chromosomes are being drawn toward the center. Fig. 13 and 18, *a*, are taken from cells in this stage.

In Fig. 19 we have a drawing made from a smear preparation. Again appear two rings, straight and bent crosses and rods. The chromosome marked *u* has its ends crossed and thus forms a modified ring. I think that this is a result of the pressure used in spreading the cells on the cover, as I saw it on no other slide. While the smear method shows the chromosomes well, in none of my preparations so made does the spindle appear and

so it is difficult to fix the exact stage of development of the cell. The chromosomes in most smear preparations were irregularly distributed in the cell, yet a few showed them in a kind of equatorial plate. I can confirm what Sinéty (28) has said about the metaphase: "A parlèr rigoureusement le métaphase néiste pas dans cette catégorie de cellules" (first spermatocyte). In many cells of first spermatocyte there is really not a metaphase, that is, there is no stage in which all the chromosomes lie in one plane at the equator of the spindle, but as some are entering others have separated and are passing to the poles. This is partly illustrated by Fig. 17, but others of my drawings which I have not been able to put among these figures show this difference of arrival at the equatorial plate region very distinctly. Some cells show a good metaphase (Fig. 14).

Fig. 16 and 17 show the division of the ring which breaks into two semicircles at the equatorial plane. Fig. 17 shows the *u*, or *v*, shaped chromosomes described by so many writers in the anaphase of the first maturation division.

The ordinary chromosomes in the semiresting stage were described above while speaking of the accessory.

Attention should be called to the difference of shape of the chromosomes as they appear in Figs. 31 and 34 or 35. While in the former we have the bent rods quite numerous, in the latter they are mostly straight rods. The spermatogonia are curved less than those in the second spermatocyte and more than those of the spermatid.

Comparison of Results and Discussion of Literature.

As indicated above Sinéty (28) and McClung (18) have described and discussed the behavior of the accessory chromosome in the spermatocytes of the Orthoptera. Both papers appeared after my own principal results were obtained. I can hence add independent, confirmatory evidence of its failure to divide in the first spermatocyte mitosis and its division in the second and the resulting distribution to only two of the four spermatids. I have not been able to confirm McClung's observation of a spireme condition of the accessory in the growth period.

From the observation of the peculiar L chromosome in *G.*

assimilis, I very much question the correctness of Sinéty's interpretation of similar appearances in *Leptynia.* But my own results show too great differences in detail between species of one genus for me to *deny* the results of another worker on a different family without seeing the form he studied.[1]

I can add the following new facts concerning the behavior of the accessory chromosome. In the first spermatocyte spindle I have a greater variation in the position in which the accessory may be found. Beside the positions described by the above writers it may be in the equatorial plate region and as I believe constricted by the cleavage of the cell. That it is not a normal division of the accessory chromosome is proven by the fact that the parts separated may be unequal. It is simply a mechanical separation of the chromatin mass.

The behavior of the accessory chromosome in the semiresting stage is parallel to conditions which Sutton (29) has described in the spermatogonia of *Brachystola.* Sutton finds that the accessory always has its own vesicular wall and does not form a part of the regular nucleus. In *Gryllus* the accessory has its own vesicle in the semiresting stage between the first and second spermatocytes. The accessory here shows its independence in the part of the germcycle in which it has not been described, and so it offers additional proof of its own individuality, hence of *the individuality* of the chromosomes.

In the variety of shapes found in the first spermatocytes I do not claim to have anything new. But in interpreting the different forms as distinctive of individual chromosomes, I have assumed *a new view point.* Anyone following the investigations on germcells since Weismann (33) postulated a reduction division and Flemming (10) distinguished between heterotypic and homotypic mitoses will find a great number of shapes of chromosomes described. Some of these are characteristic of certain species. Other species have a great variety of shapes in the same cell. The efforts of the workers who have found these different shapes

[1] Dr. McClung informs me privately, since the above was written, that he has found appearances in an Acrididæ which indicate that the accessory chromosome may unite with an ordinary chromosome forming an L-shaped mass; and so Sinéty's interpretation is probably correct. But the accessory *is not* a part of the L-shaped chromosome in *G. assimilis.*

have been directed toward reducing them to a single type or form, or to show that all accomplish the same purpose and that the variations are only chance differences or certain stages in the development of the tetrad or stages in the first spermatocyte division.

It is impossible to discuss within the limits of this paper all the works that have described and discussed a variety of chromosome forms in the spermatocytes and ovocytes. For such discussions I would refer the reader to Wilson's "Cell," p. 264 ff., and Korschelt and Heider's "Entwickelungsgeschichte," p. 572 ff.

Wilson's interpretation of these different shapes is evident from this quotation: "But even in cases where the chromatin does not condense into actual tetrads these bodies are represented by chromosomes in the form of rings, crosses and the like, which are closely similar and doubtless equivalent to those from which actual tetrads arise, and present us with the same problems. With a few apparent acceptions described hereafter, the tetrads, or their equivalents, always arise by a double division of a single primary chromatin rod or mass." Wilson then discusses the various maturation divisions and chromosome shapes under:

(*a*) "Tetrad formation with one longitudinal and one transverse division," naming Henking's, Vom Rath's, Haecker's, *et al.* results.

(*b*) "Tetrad formation with two longitudinal divisions," Van Beneden and Boveri on Ascaris.

(*c*) "Tetrad formation by conjugation," Wilcox and Calkins especially.

In these cases the rings, etc., occur in the prophases.

"Reduction without tetrad formation" occurs where there is no resting stage and here "the equivalents of tetrads," the rings, crosses, etc., appear. Again, the divisions are longitudinal and transverse, found mostly in Invertebrates, or double longitudinal, found mostly in Vertebrates and in many plants.

Korschelt and Heider classify the various mitoses under "*eumitotic*," or double longitudinal, and "*pseudomitotic*," or one longitudinal and one transverse — a reduction division. After discussing very many papers giving the various forms of chromo-

somes described, and the interpretation that has been put on them, they make the suggestion (p. 591) that many of the chromosome forms are artefacts, "dass es sich bei manchen von ihnen um Kunstproduckte handeln möchte, wie sie durch die Conservirung hervorgerufen werden."

From the two excellent reviews of the literature referred to above it will be seen that all attempts at an explanation of the various shapes aimed at proving that there were two longitudinal splits of the chromosomes and hence two equational divisions, or a longitudinal split and a transverse split, hence an equational and a reductional division. Reduction is the question around which the whole discussion centers.

Montgomery's (25) observations on the salamanders show that in these forms there is a side-to-side union of the chromosomes in synapsis. The Schreiners (27) have observed the same fact in some of the lower fishes. If this proves true for the vertebrates in general, and if Farmer and Moore are correct in interpreting one of the longitudinal splits in plants as the line of union of two chromosomes, then the contradiction between Korschelt and Heider's eumitotic and pseudomitotic division is removed. The question as to which is the equation and which is reduction division has largely lost its importance, not because they have lost their significance as Wilcox (34) put forth, but because it is shown that there is an equation and a reduction division whatever may be the appearance of the chromosome in the prophase of the first maturation division (see Montgomery (26)).

I wish now, after having referred to the above general reviews, to discuss several papers more in detail because of their interest from my special view point.

Griffin's (12) work is taken by Korschelt and Heider (14) as a typical case of their "post-reductional" divisions. He gives careful descriptions of minute details. In the prophase he figures fourteen different shapes in the text and describes them as "rings, crosses, double rods, and apparently homogeneous rods variously coiled and bent." On page 607 he says: "Despite the varied forms presented during the prophase the chromosomes of the equatorial plate exhibit considerable uniformity. Hence the various prophase forms must in some manner be convertible into

a uniform type of metaphase figure." Then in the metaphase he tries to reduce all to the cross type. But I do not think his drawings bear out his contention (see his drawings of *Thallasema* Figs. 12, 13 and 14 of Plate XXXI). Especially in Fig. 13 I do not see how he will get all to the cross type.

We have the following recent papers on the Orthoptera: McClung (17) indicates various shapes in his figures of the early prophases and says: "But despite the multiplicity of their forms these precursors of the chromosomes are all referable to a common type." This is a doubly split rod. Crosses in prophases are the result of the gliding together of the chromatids, or parts of the split rod. As a result of the concentration of the chromatin "the chromosomes in the nuclear plate appear to be simple homogeneous bodies." But they have the shape of rods, crosses, v's and rings. These McClung explains as the result of different views of the crosses, and the gliding of the chromatids or the bending of the arms of the crosses.

The interesting fact here is that while Griffin and most of the earlier writers tried to find that the chromosomes in metaphase either were all the same shape or could be reduced to the same type, McClung finds the various shapes in the metaphase, and rings divide as rings and crosses as crosses.

McClung (18) describes rings, crosses, figure 8, etc., in the prophase in the Locustidæ: "After concentration, while all trace of internal structure" (chromomeres and splits) "are gone, the general outline is retained and the crosses and rings of the early stages are still even up to the metaphase crosses and rings."

Sinéty figures a great variety in the Phasmidæ, Locustidæ, Acrididæ and Gryllidæ. The different shapes in the metaphase he explains by the different manner of insertion of the mantle fibers. This may be "median, subterminal or terminal." But this gives no reason for their shapes in the prophases.

Schreiner (27), A. and K. E., have described very recently in a hag-fish, *Myxine glutinosa*, and a dog-fish, *Spinax niger*, various forms of chromosomes in the prophases. In *Myxine* the chromosomes are concentrated into round or polygonal bodies, but "in a few cases they show in this stage plainly a ring form."

There is a pair of very large chromosomes in the spermatogonia and a single very large one in the spermatocyte. In *Spinax* there are graded size differences of chromosomes which they compare with Montgomery's and Sutton's results. They think the number of large chromosomes and small ones is constant but have not proven this. The large chromosomes form rings. The small ones form before mitosis mostly rods, seldom rings—"häufig Stäbchen, selten Ringe." In the equatorial plate the chromosomes retain their shape.

Here we have then from the recent papers, McClung, Sinéty and Schreiner, evidence that the shapes found in the prophases are still found in the metaphase. Their drawings do not show as many different forms in the metaphase as in the prophases, but some are probably hidden. In *Gryllus* all the various shapes in a cell are seen to best advantage in a late prophase, but I believe all the shapes are still to be seen in metaphase. A careful comparison of my Figs. 11 and 12, late prophases, with Figs. 14 and 16, metaphase will prove that the same shapes are found in earlier and later stages. The different kinds of attachment of the mantle fibers can not account for different forms in the prophases, although it may in the metaphase. Fig. 11 is very instructive. The chromosomes are still granular and rough in contour yet we find two rings, the number probably found constantly, and two crosses, a number frequently observed.

That these various shapes are artefacts as Korschelt and Heider (14) suggest, is made very doubtful by the fact that they are shown by my material fixed in very different reagents. They are shown in my sections and in my smear preparations. Besides the great army of investigators have used all kinds of fixatives and yet have found them.

That they are not simply stages through which the chromatin must pass to get into the *v* or horseshoe shape of the anaphase as Lebrun (15) has assumed, is combated by the long continuation of the same form from the early prophase to the anaphase. To reach such an end would need only *one* type of chromosome form in a single nucleus at any one time. This is contrary to the observation of the majority of workers who have observed chromosome differences.

That the shape is not a mere happening so, a ring now, and a cross then, is met, in part at least, by the probability of the constancy of the number of rings found in the various cells. I could see no way of testing the constancy of the other forms, such as the cross.

Taking all these observations into consideration it seems to me the best hypothesis that we can propose to explain the various forms assumed by the chromosomes in the prophases and metaphase of the first spermatocyte is to say *that these various shapes are an expression of the individual characteristics of the various chromosomes.* They are a proof of the individuality of the chromosomes. I would repeat in the way of emphasis that Boveri has found a difference *in function*, Sutton a difference *in size* and I a difference *in form* in the chromosomes of the germ cells.

That these observations will be found to have a wider application I would predict from the many indications I find in literature especially the results on *Ascaris.* But in many species as in *G. assimilis* the form of the chromosomes is not varied enough to establish a constancy of any one form, just as size differences can probably not be found in all species. Some other species will probably prove much better to establish morphological differences in the chromosomes than *G. domesticus*; yet I consider this material very good for the purpose. I trust that other workers who find a great variety of chromosome shapes will examine their material from this view point.

That the hypothesis, or theory as Boveri (6) would call it, has received an unfortunate name Wilson points out in his "Cell," p. 299. But I do not believe that many cytologists think that chromosomes persist as individuals. Nor do I believe that we generally think of a "continuity of the chromatic substance" as Haecker (13) supposes (see page 217). Nor is he the first to suggest that the continuity may rest upon the achromatic substance as shown in the quotation below.

This passage quoted from Boveri (4) I think gives an idea of what the advocates of the hypothesis mean by the individuality of the chromosomes. "Ich habe dieselbe als die Hypothese von der Individualität der Chromosomen bezeichnet, weil die Gebilde, die wir als selbstständige Stücke kennen, den Namen

"Chromosomen" führen, und die nächstliegende Annahme war nach den Befunden von Rabl und mir in der That die, dass jedes Chromosoma als solches in ruhendem Kern fortbestehe und nur seine form verändere. In letzter Instanz aber fordert die Hypothese nichts anderes als einen genetishen Zusammenhang zwischen je einem der aus dem ruhenden Kern hervorgehenden Elemente mit einem bestimmten der in die Bildung des Kernes eingegangenen. Was von dem Chromosoma als selbstständiges Gebilde übrig bleibt, ist für die Hypothese an und für sich gleichgiltig. Es mag unser hypothetisches Individuum z. B. die färbbare Substanz völlig verlieren und sich erst wieder bei der nächsten Teilung mit ihr beladen; ja es mag in gewissen Zellen nur ein mit unseren Mitteln gar nicht nachweisbares Teilchen von jedem Chromosoma übrig bleiben um als Bildungscentrum zur Entstehung der neuen Chromatin schleife Veranlassung zu geben: jedenfalls ist die Annahme eines genetishen Zusammenhanges je eines bestimmsten Chromatinsegmentes mit einem bestimmten der vorher sichtbaren die weitaus bestbegründete Annahme zur Erklärung aller in Betracht kommenden Erscheinungen und vor allem der bei den Kernteilungen zu beobachtenden normalen und abnormalen Zahlenverhaltnisse." It is the genetic relation of which most of us think when speaking of chromosome individuality. Such an individuality would be supported by the constancy of the appearance of a certain shape in a bivalent chromosome. If two pairs of chromosomes after conjugation form rings in every cell of a first spermatocyte generation they will form them in succeeding first spermatocytes and that means that there is a genetic relation.

It is not wise to theorize much on the importance of a hypothesis based on so few observations, but a few words indicating its meaning might not be amiss.

A constant morphological difference would strongly support Boveri (5) and Sutton (31) in their surmise that the chromosomes play different rôles in development. Sutton (31) makes this statement: "There is reason to believe that the division products of a given chromosome in *Brachystola* maintain in their respective series the same size relation as did the parent element; and this taken together with the evidence that the various chro-

mosomes of the series represent distinctive potentialities, make it probable that a given size-relation is characteristic of the physical basis of a definite set of characters." In the above sentence I should like to substitute *form* for "size-relation" and *Gryllus* for *Brachystola*. A difference in the shape of the chromosomes indicates the fact that they form the physical basis of different sets of characters. A difference of shape supports Boveri's supposition of a difference of "rôle of chromosome" even more strongly than a difference of size. But Boveri's method of study, multipolar spindles, can not be applied to Orthoptera. To me it seems probable that the test, if we can get any, must come from hybrid germ cells. Sutton (31) has shown that "the phenomena of germ-cell division and of heredity" as expressed by Mendel's laws "are seen to have the same essential features." Moenkhouse's (21) and Metcalf's (20) results teach us that forms with differently shaped chromosomes can be crossed. If now we can raise such hybrids to sexual maturity, we can probably get light on the "purity of the germ-cells" as well as on the meaning of the various shapes of the chromosomes.

Montgomery (26) criticizes Sutton (31) and says that the combinations of the paternal and maternal chromosomes in the fertilized egg do not follow the Mendelian ratio. In sustaining this position Montgomery says the Mendelian ratio can hold only in cases where there are but two chromosomes in the fertilized egg. For the case of four chromosomes the relation would be 1 : 14 : 1 instead of 1 : 2 : 1, and for twenty-four chromosomes 1 : 16,777,214 : 1. Montgomery must have entirely misunderstood Sutton or the Mendelian principle. Mendel found that *a pair of alternative characters* followed in self-fertilized hybrids the ratio of 1 : 2 : 1. Sutton found the same ratio for a *pair* of homologous chromosomes. Sutton also found in a form that had twenty-four chromosomes there could be 16,777,214 different combinations of chromosomes in the gamete. To take a concrete example in hybrids having twenty-four chromosomes a pair of alternative characters ought to appear in the second generation hybrid in the proportion 1D : 2Dr : 1r, but only one out of 16,777,214 of such hybrids should show *all the characters* belonging to one of the pure ancestors only and none of those of the other. Probably Dr.

Montgomery meant to explain his position and correct the error, when in his criticism of Haecker in the Zool. Anz. of June 14 he said: "Indeed, my position is exactly that of Sutton who argued that it would be purely a matter of chance as to which daughter cell a particular chromosome would enter."

As indicated above, a brief criticism of Voinov's (32) interpretation of the double nucleinic body will be given. If his interpretation were correct that the nucleolus contains all the condensed chromatic matter, he is wrong in claiming that this has been described only in ovogenesis. I would refer him to Blackman's (1 and 2) works on Myriapods.

But he is wrong in saying that the nucleolus and accessory always approach each other in the later growth period. They may be close together in the early part of the growth period, as shown in Fig. 6. The nucleolus may become pale and disappear far removed from the accessory. The latter does not disappear. Voinov says, as Blackman (1) has found in Myriopods, that the nucleolus acts as a reservoir for the chromatin during the rest stage. I doubt this for *Gryllus*, because the nucleolus is not largest when the chromatin is least apparent in the spireme. Voinov (32) was probably misled by his staining results. One lot of my *G. domesticus* shows just such conditions that would lead one to think the chromatin in the growth period is stored in the nucleolus, but all my other lots refute this.

In the "Observation" above I criticised Montgomery's (22) description of synapsis. This is a criticism of the stage as he described it in *Pentatoma* and by implication in *Gryllus*. I do not find a massing as he described it in "Synapsis," but rather a looping as described in "Post-synapsis." This is the stage that Montgomery (25 and 26) has emphasized in his later papers. The ovocytes in the Gryllidæ show the loops crowded to one pole of the nucleus, as Montgomery has described in *Peripatus* and certain salamanders. The loops in the crickets are also present in the reduced number and are probably formed, as Montgomery has suggested, by the union of the parental chromosomes into pairs. He finds that the closer union is at the central pole, but my material shows more free ends at the central pole (see Fig. 36). I should conclude that the closer union is at

the distal pole. The spermatocytes are so small that conditions there are much more difficult to decipher. Further study will probably clear up this matter and reveal the method of formation of the various chromosomes in the growth period.

It gives me pleasure to express my gratitude to Dr. Frank R. Lillie for reading this manuscript, as well as much for helpful encouragement during the progress of the work.

HULL ZOÖLOGICAL LABORATORY,
UNIVERSITY OF CHICAGO,
August 6, 1904.

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EXPLANATION OF PLATE I.

All drawings were carefully outlined with a camera lucida and the details filled in afterwards. For all the figures except 22 a Leitz $\frac{1}{16}$ objective and a Zeiss ocular 18 was used. The reduction is approximately $\frac{2}{5}$ giving a final magnification of 2,900 diameters.

All the figures are taken from *Gryllus domesticus* except 22 and 24 from *G assimilis* and 36 from *Scapteriscus didactylus.*

The drawings are all numbered as nearly as possible in the order of the stages of development represented. Figs. 18, 19 and 22 are exceptions.

In all figures $x =$ accessory chromosome, $n =$ nucleolus.

FIG. 1. Polar view of equatorial plate of a spermatogonial division. Accessory bent into a U.

FIG. 2. Idem. Accessory stretched out. The numbers indicate the probable pairs of chromosomes.

FIG. 3. Side view of spermatogonial spindle, the chromosomes just separating.

FIG. 4. Early stage of growth period. Accessory adherent to one side. The cell wall as in many other cases is not drawn as a definite line, because it is in many cases not visibly differentiated by the stain and the exact limits between cells can not be made out.

FIG. 5. Later growth period. The chromatin in loops of spireme.

FIGS. 6 AND 7. Later stages of the spireme. The chromatin still more or less in loops. The accessory and nucleolus in juxta-position. Accessory in 6 lies in reality against wall.

FIG. 8. Later than above. One chromosome forms a ring. Accessory and nucleolus at almost opposite sides of the nucleus.

FIG. 9. Fragment of cell showing longitudinally split chromatin rods.

FIG. 10. Chromatin somewhat more diffused. Accessory and nucleolus lie together, but latter very pale in color.

FIG. 11. Late prophase, the accessory dense and with smooth contour. Ordinary chromosomes somewhat granular and with ragged contour. Chromosome 6 was drawn from next section.

FIG. 12. Polar view of very late prophase or metaphase of first spermatocyte spindle. Chromosomes lie at different levels.

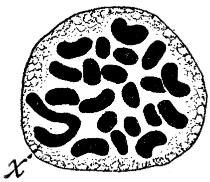


Fig. 1

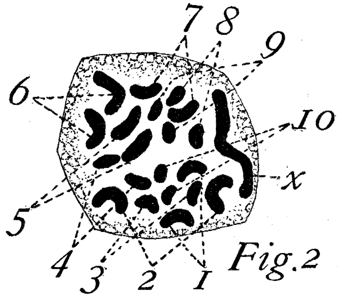


Fig. 2

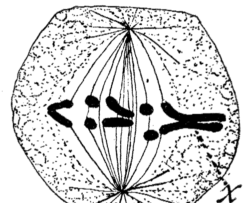


Fig. 3

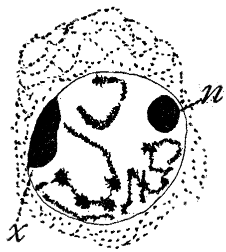


Fig. 4

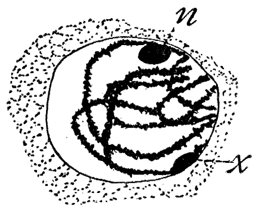


Fig. 5

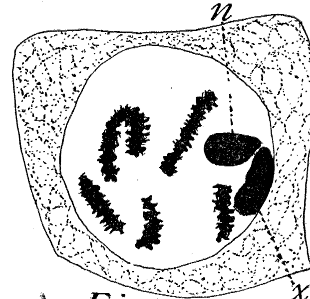


Fig. 7

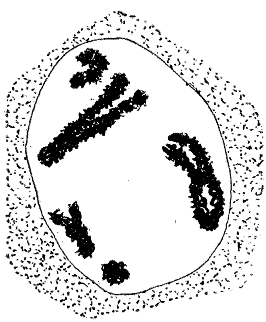


Fig. 9

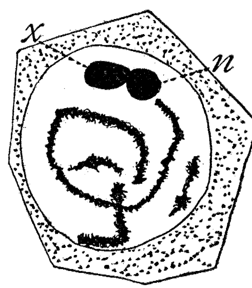


Fig. 6

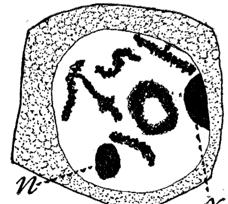


Fig. 8

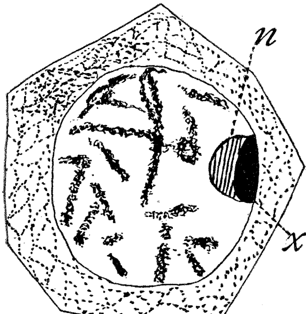


Fig. 10

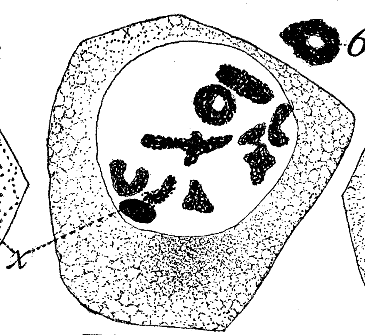


Fig. 11

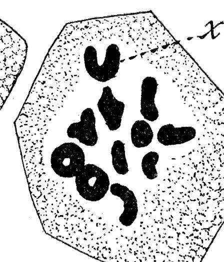


Fig. 12

Explanation of Plate II.

Figs. 13, 14 and 15. Lateral views of the spindle. Chromosomes show various shapes.

Fig. 16. Lateral view of chromosomes showing one ring broken on one side.

Fig. 17. Metaphase showing a ring separating.

Fig. 18. Groups *a*, *b* and *c* show different shapes of chromosomes. Each group was drawn from a single nucleus. Group *a* is from very late prophase; that is, not all the chromosomes have reached the equator of the spindle. The third chromosome in *b* is the accessory.

Fig. 19. A drawing made from a smear preparation showing the various shapes. The chromosome marked *u* has the ends crossed and may be the result of pressure on the nucleus.

Fig. 20. Anaphase showing accessory nearer one pole and outside the spindle.

Fig. 21. Later anaphase, accessory caught in the center and showing constrictions.

Fig. 22. Telophase showing accessory separated by cell walls. Magnification, 1,860 diameters.

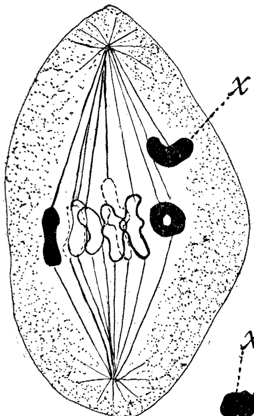
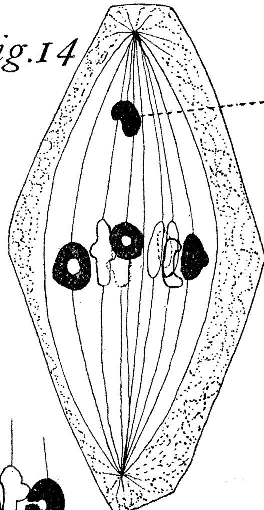


Fig.13

Fig.14


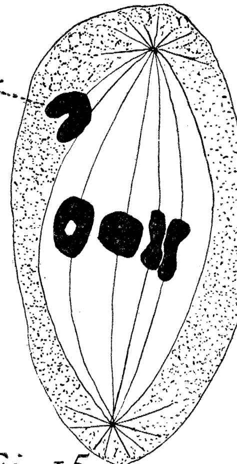


Fig.15

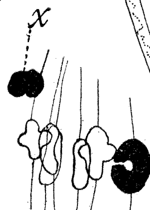


Fig.16

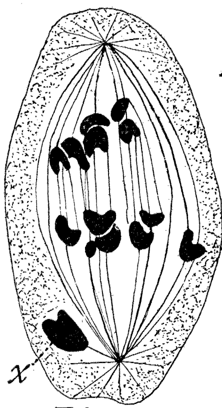


Fig.20

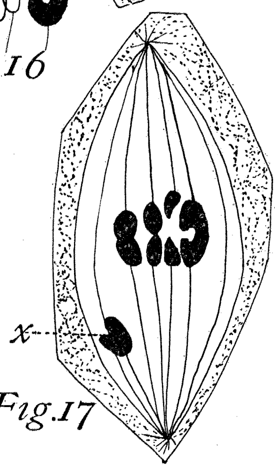


Fig.17

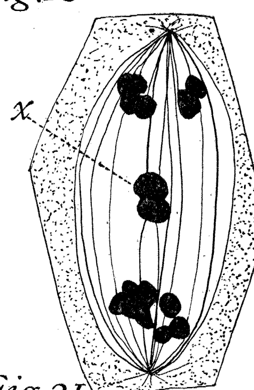


Fig.21

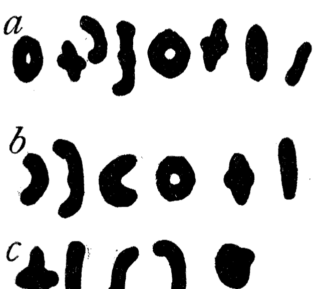


Fig.18

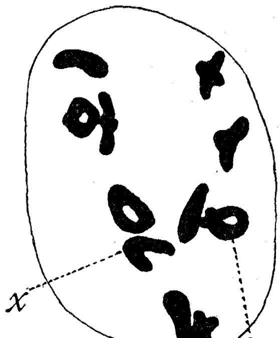


Fig.19

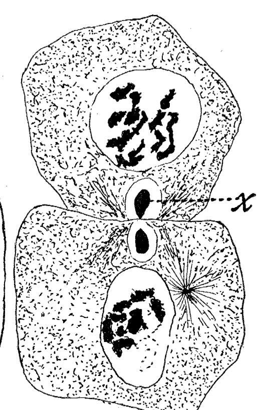


Fig.22

EXPLANATION OF PLATE III.

FIG. 23. Late anaphase. Centrosome divided. Accessory lagging.

FIG. 24. Late anaphase. Spindle between the accessory and the ordinary chromosomes.

FIGS. 25, 26 AND 27 show conditions of the semiresting stage. Accessory has its own vesicle. The ordinary chromosomes are vesicular in 25 and 26.

FIGS. 28 AND 29. Polar views of second spermatocyte spindle. Chromosomes crowded.

FIG. 30. Side view of second spindle.

FIG. 31. Polar view of second spindle. Chromosomes not so crowded.

FIGS. 32 AND 33. Anaphases of second spindle; 32 shows accessory.

FIGS. 34 AND 35. Polar views of anaphases of second spindle. The twin cell of 34 shows the other part of the divided accessory.

FIG. 36. Ovocyte of *Scapteriscus*. Synapsis stage.

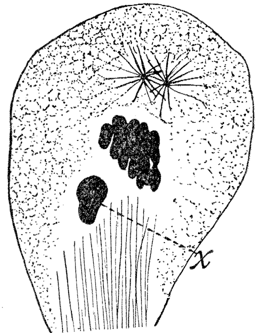


Fig. 23

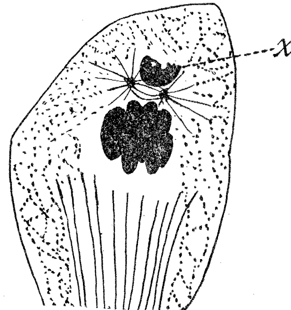


Fig. 24

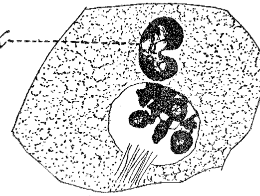


Fig. 26

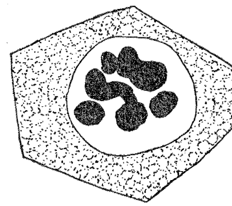


Fig. 28

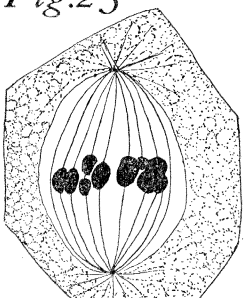


Fig. 30

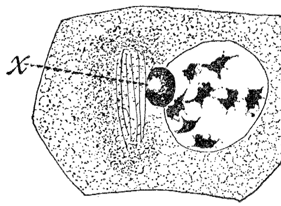


Fig. 27

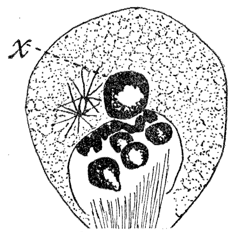


Fig. 25

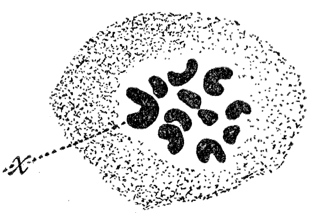


Fig. 31

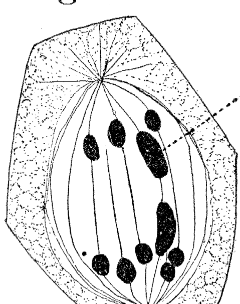


Fig. 32

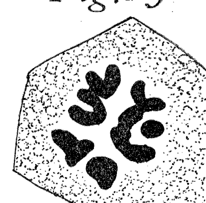


Fig. 29

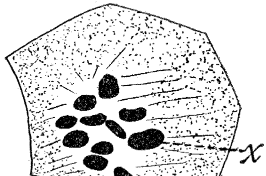


Fig. 34

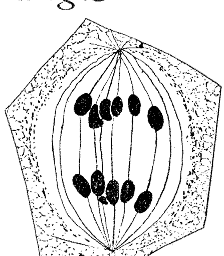


Fig. 33

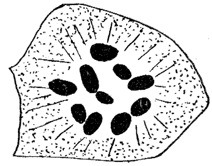


Fig. 35

Fig. 36